ATTORNEY DOCKET NO.: **215105.01500** PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	:	Examiner: Melanie J. Yu
Cary James MILLER, ET AL.	)	
	:	Group Art Unit: 1641
Application No.: 10/658,529	)	
	:	Confirmation Number: 5356
Filed: September 10, 2003	)	
	:	
For: IMMUNOASSAY DEVICE WITH IMMUNO-REFERENCE ELECTRODE	) :	May 21, 2008

MAIL STOP FEE AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF INVENTORS UNDER 37 C.F.R. §1.131

Sir:

We, Cary James Miller and John Lewis Emerson Campbell, whose full post office addresses are 19 Windfield Crescent, Ottawa, Ontario, Canada K2M 2B7 and 145 Charmont Way, Woodlawn, Ontario, Canada K0A 3M0, respectively, hereby declare and say as follows:

1. We are the only inventors of the subject matter disclosed and claimed in the above-identified patent application, namely, U.S. Patent Application No. 10/658,529, filed on September 10, 2003. In preparing this Declaration, we have reviewed the subject patent application, the January 25, 2008 Office Action, and the Response being submitted concurrently herewith.

2. We jointly conceived and reduced to practice the subject matter of at least the inventions recited in Claims 1, 2, 5-7, 9-12, 69, and 70 of the subject patent application prior to the December 3, 2002 filing date of U.S. Patent Application Publication No. US 2004/0106190 A1 to Yang, et al. As factual evidence of this, the following facts are entered with supporting documentation.

3. Prior to December 3, 2002, we had invented certain novel and inventive improvements in an immunoassay device with an immuno-reference electrode, for which we believed patent protection should be obtained.

4. Attached as Exhibit A is an excerpt of a memorandum having a date prior to December 3, 2002 that fully describes the subject matter claimed in the present application, thereby demonstrating conception of the subject matter claimed in the present application.

5. Attached as Exhibit B is an excerpt of a document entitled "The iSTAT Immunoassay Platform" that describes an operational system that embodies the subject matter claimed in the present application. In particular, as described at pages 79 and 80 of this excerpt, an algorithm and experimental data are provided, thereby demonstrating an actual reduction to practice of the subject matter claimed in the present application.

6. Attached as Exhibit C is an entry from a laboratory notebook that shows a photograph of a system that embodies the subject matter claimed in the present application. In particular, the photograph shows a pair of immunosensors, including a first sensor, labeled "TnI", which acts as the analyte sensor, and a second sensor, labeled "HSA", which acts as the reference sensor. This photograph corroborates the demonstration of an actual reduction to practice of the subject matter claimed in the present application.

7. We declare that this memorandum, document excerpt, and laboratory notebook entry evidence our conception and reduction to practice of the subject matter claimed in the present application as of a date prior to December 3, 2002.

8. It is therefore respectfully submitted that the present patent application claims an invention which was conceived and actually reduced to practice prior to December 3, 2002.

9. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declared and signed:

Cary James Miller	20 May 2008
Cary James Miller	Date
	20-May-2008
Witness	Date
	20 MAY 2008
John Lewis Emerson Campbell	Date
	20 MAY 2008
Witness	Date

EXHIBIT A

202 339 8260

MEMO : NEW PATENTABLE CARTRIDGE FEATURES

DATE : [redacted]

FROM : Graham Davis

TO : Ybet Villacorta (KMZR)

COPY : Mike Zelin, Cary Miller, Celine Bisson & John Campbell

FILE : Bloodentryclaims1

In advancing the development of the immunoassay and coagulation cartridges we have conceived of new features that appear novel and worth patenting. With regard to the immunoassay features additional details are in the I-STAT Immunoassay Platform document. Details of the coagulation features related to blood collection are covered here.

With regard to filing an application, I think the most economical approach is to add all the new disclosure and any new claims to the pending immunoassay cartridge application (.0800) and incorporate by reference the pending .0600 coagulation application. This is feasible as the new developments from the coagulation program are not test-specific, but relate to blood entry and are applicable to all cartridge assays. Given the range of new subject matter for claims, we will probably get a restriction notice from the examiner.

<u>New blood entry sealing concept</u>

In the immunoassay disclosure, this concept is referred to as "Moby", see section 2.

Problem solved: Prevention of blood being forced beyond capillary stop element, which does occur in a limited percentage of cartridges with the standard snap-closure structure during closure. Specifically, when a cartridge is filled to slight excess, activation of the snap-closure can provide a force that breaks the capillary stop. This is not an issue for the tests we currently sell which measure bulk concentrations and are thus not volume dependent. However, this is an issue in immunoassay applications where accurate metering of the sample is required as we capture analyte from a fixed sample volume, i.e. the sample volume is a variable that has a fixed value in the algorithm that calculates the analyte concentration from the sensor signal.

In the cartridge, the metered volume is designed to be from the bladder air entry point to the capillary stop, which is designed to be 10uL. If the capillary stop is broken additional blood beyond the capillary stop will also be assayed, i.e. the total assayed volume could

be as much as 12uL. This can lead to an erroneously high result. The Moby clip obviates this problem by sealing the cartridge without inadvertent displacement of the sample breaking the capillary stop. This leads to statistically better quality results from the system.

Apparatus invention: A sealing means for a blood collection device comprising a housing, a substantially flat target orifice, an internal blood holding chamber, an external overflow chamber, an internal capillary stop element and an internal conduit, where said sealing means is movable in the plane of the orifice to provide a shear force to blood in the region of the orifice where any blood above the plane of said orifice is displaced towards the overflow chamber and any blood below the plane is sealed within the holding chamber.

Method invention: A method of sealing a blood collection device comprising a housing, a substantially flat target orifice, a sealing element, an internal blood holding chamber, an external overflow chamber, an internal capillary stop element and an internal conduit, where in a first step a blood sample is applied to said orifice and fills said holding chamber up to said capillary stop, where in a second step said sealing means is moved (manually actuated) in the plane of the orifice to provide a shear force to blood in the region of the orifice where any blood above the plane of said orifice is displaced to the overflow chamber and any blood below the plane is sealed within the holding chamber, where said shear force is (substantially) less than the force required to force the blood past the capillary stop and into said conduit.

Dependent claims:
-where the orifice is circular or oval
-where the diameter of the orifice is in the range 0.5-5mm (or perimeter 1-15mm)
-where the region around the orifice is hydrophobic, hydrophilic, lipophilic etc.
-where the region around the orifice is an adhesive capable of forming a seal with the said sealing means
-where sealing means locks into a sealed position
-where locking occurs when a tooth on the sealing means enters a slot on said housing
-where said housing comprises a groove for directing the motion of said sealing element in the plane pf said orifice
-where said sealing element comprises an upper facet and a lower facet, where said upper facet provides a sealing surface and said lower facet moves in said groove
-where said sealing element, in moving from said initial position to said sealed position, said lower facet flexes to provide a downward force to said upper facet, to seal said orifice.

Inventors: Miller, others?

<u>Immunoassay reference sensor</u>

In the immunoassay document, the concept of an immunoassay reference sensor is discussed in section 3.1 (see page 42 and 52).

Problem solved: The signal from an immunosensor is intended to be derived solely from the formation of a sandwich between an immobilized antibody, the analyte and a labeled second antibody, where the label reacts to form a detectable product (1).

Surface-Ab1~analyte~Ab2-Enzyme; Enzyme + S → P (1)

However, it is possible that the second antibody binds non-specifically to the surface and is not removed in a wash step (2), thus giving rise to a portion of the total product that is not a function of the analyte.

Surface~Ab2-Enzyme; Enzyme + S → P (2)

While the immunosensor is optimized to limit non-specific binding using known blocking agents, here we intentionally create a second sensor which acts as a single-use reference sensor intended to give the same degree of non-specific binding that occurs on the immunosensor. Thus by subtracting the signal at the reference sensor from the signal at the immunosensor, the non-specific component of that signal can be removed.

The reference sensor is preferably the same in all respects as the immunosensor (dimensions, latex particle coating, metal electrode composition etc.), except that the capture antibody for the analyte (e.g. cTnI) is replaced by an antibody to a plasma protein that naturally occurs in all (normal and pathological) samples at a high concentration. Desirable examples are albumin, fibrin and IgG fc region.

In an alternative embodiment, the immunosensor may be coated with a combination of plasma protein antibody and troponin antibody. Here the manufacturing ratio needs to be controlled to ensure that the coated particles have suitable amounts of both antibodies. It has been found that with an albumin antibody concentration on the microparticle of less that 20% of the troponin antibody concentration, the slope of the troponin assay is not affected.

Apparatus inventions: A reference immunosensor, comprising a reference sensing surface with a layer of antibody to common human serum protein covering at least a portion thereof.

A reference sensor for an immunosensor, comprising a reference sensing surface with a layer of antibody to a common human serum protein covering at least a portion thereof, said human protein selected to be commonly present in a blood sample at a concentration sufficient to bind substantially all of the available antibody.

A combination of an immunosensor and a reference immunosensor, comprising a reference sensing surface with a layer of antibody to a common human serum protein

EXHIBIT B

The i-STAT Immunoassay Platform

Cartridge Design and Function
As of [redacted]

Arian Amirkeyvan
Cary Miller
John Campbell
Meghan Hawkes
Pamela Frank
Shannon Lobin
Tamara McCaw
Zhen Yang

4. i-STAT Immunoassay Software and Algorithm for Calculation of [TnI]

Development of the immunoassay cartridge has benefited from the existence of the i-STAT "Sequence Software". Sequence software , created (D. Opalsky) several years ago, is essentially a macro language for control of analyzer motor motions and data acquisition engines. This means that non-software specialists can create software with customized motor motions and data acquisition schemes. The rudiments of sequence operation can be gleaned from the document: SequenceManual.Doc.

4.1. The Immunoassay Cartridge Cycle

As of this writing, the immunoassay cartridge cycle is executed in series 300 analyzers loaded with CMX4096R.BIN software. The remainder of this section will consider the sequence cycle as executed in this version. For the sake of brevity, only general features of the cycle will be discussed here – the reader is referred to the source code (CMX4096R_g_sequen.C) for further details. Cartridge data may be viewed in WinISD using the initialization file CMX4096R.INI

4.1.1 Pre-Sample Push / Pre-Sequence

Upon insertion of the cartridge, the analyzer mechanism makes contact with and identifies the cartridge, conducts battery and other instrument checks, and initiates and completes the thermal cycle. When the contact pins come off the shorting bar, the motor is slowed from 60% to 25% of its calibrated speed. This is important because the subsequent motion of the mechanism causes the sample to be pushed from the holding chamber through the sample cap stop and into the cover pre-push conduit. It is important that this fluid motion be effected slowly and evenly so as to ensure precise delivery of the sample to the sensor channel without entrainment of bubbles or significant alteration of sample volume. The motion stops with the plunger 0.003" above its calibrated position – this pushes the sample fully from the sample chamber, but not so far as to contact the sensor chips during the bang-bang (thermal cycle). The push pin makes contact with the cover paddle during the second

motor motion causing the analysis fluid to be dispensed from the calpack into the analysis fluid conduit/holding chamber. It is at this point that control over the cycle is accessible through sequence commands.

4.1.2 Sample Push and Capture

Upon entry to the sequence, the temperature set-point is increased to 47 degrees, and data channels for storage of motor back emf (BEMF) and hematocrit conductivity are initialized. The sample is pushed at 25% of calibrated speed until its arrival at the Hct sensor is detected. A period of 8 sec is allowed for this push – if the sample does not arrive within this time, a leak and consequent loss of air-bladder gain is indicated, and the cycle is terminated with SeqErrorCode 1001. The product version of the software is anticipated to have a tighter spec on the arrival time with a check that the sample has not arrived prior to the push (flags a used or overfilled cartridge). Additionally, the product software may check that the sample contains no bubbles during its initial pass over the Hct bars.

The sample push is continued until the sample passes the Hct sensor whereupon the motor speed is reversed and the capture oscillation/mix commences. The capture is currently executed at a speed 100% of the calibrated motor speed – the duration of the capture is currently 7 minutes for a total test duration of about 10 minutes. The BEMF and conductivity are sampled during cycles 50 through 55 and 150 through 155 for post-run QC of the mixing data – the product version will likely involve a running QC during the capture phase so that an abnormal mix will trigger an error condition as early as possible.

Phase	**Data Channels**
Sample Push & Capture	Channel3 (WinISD Channel4) = BEMF Channel4 (WinISD Channel5) = Conductivity

BEMF and conductivity data are recorded at a resolution of 20 ms per data point during sample push and capture.

Lock, Wash & Analysis	Channel3 (WinISD Channel4) = BEMF
	Channel4 (WinISD Channel5) = Conductivity
	Channel5 (WinISD Channel1) = Conductivity (high resolution)
	Channel0 (WinISD Channel7) = AMP0
	Channel2 (WinISD Channel6) = AMP1

BEMF and conductivity are collected at a resolution of 320 ms per data point; conductivity during the wash is collected at 20 ms resolution on a separate channel in order to afford data for QC of the wash. Amperometric data are collected at a resolution of 160 ms per data point.

Immediately before the last two oscillations, the heaters are turned off and the remainder of the cycle is conducted at ambient temperature. During the remainder of the cycle, there are extended periods (> 2-3s) in which the sensors are dry and so in order to avoid denaturation of proteins on the sensor, heating is to be avoided. Though ALP will generate higher currents (analysis phase, see below) at higher temperature, it is observed that the currents are more precise (albeit smaller) when the analysis is conducted at ambient temperature.

4.1.3 Lock Valve Actuation

Following capture, the hybrid is set up to collect amperometric data at high gain (2 pA/bit) with the sensors poised at + 30 mV vs Ag/AgCl; BEMF and conductivity data recorders (channels denoted above) are initialized. The sample is moved slowly to the sample inlet side of the waste chamber in order to consolidate it. The current flowing is measured, compared with a fixed value (currently 1500 bits at 2 pA/bit), and if in excess of this value, the cycle is terminated with SeqErrorCode 1002, the sample is contaminated with analysis fluid. If the current level is

acceptable, the sample is pushed by means of several motor motions into the lock wick which becomes saturated thereby closing off the major vent.

4.1.4 Wash of Sensor Conduit

After a brief pause to allow the lock wick to become saturated, the motor is moved in a negative direction at 200% of the calibrated speed for a fixed distance. This causes the plunger to retract from the air bladder creating a vacuum in the sensor channel. In response to this vacuum, analysis fluid is drawn through the capillary joining sensor and analysis fluid conduits, and into the sensor conduit towards the sample chamber. A period of 2 s is allowed for the analysis fluid to arrive at the hematocrit bars. If the fluid fails to arrive, the plunger is retracted further at 25% of the calibrated speed until the fluid arrives; if fluid arrival does not occur within 4 s, SeqErrorCode 1005 is set. For development purposes, a 1005 error does not cause the cycle to abort – instead the second wash motion is engaged. If fluid arrival at the Hct bars was detected, the fluid is slowly moved off and on the Hct bars, the "wiggle". At this point, the sample is allowed to remain quiescent for 10 s. . The "wiggle" and the delay are helpful in consistently positioning the first wash fluid edge near the Hct bars. The gain profile and relaxation of the cartridge after the first wash motion can result in a variable initial wash fluid edge if this active positioning is not used. Following the delay, two further wash pulls are executed at 200% of the calibrated speed with a 4 s delay between them.

The wash phase ends with a routine that executes a series of small pulls of the fluid off the chip. During these motions, the software seeks a fixed number of consecutive measurements (currently 15) in which the hematocrit sensor is "dry" (resistance > 2500 bits). If these measurements are found, the chip is deemed to be dry, and the "MoveToDry" routine is exited. If the condition is not met within 70 pulls, SeqErrorCode 1003 is set ("possible wet sensor") and the cycle is continued. In product software, failure to meet the condition will result in a cycle abort – for development purposes, the cycle is currently allowed to continue.

During the pull to dry, it is possible for a "late" segment of fluid to be pulled over the sensors, but not so far as to be detected at the Hct bars. For this reason, after a successful move to dry, the fluid is pushed back towards the chip so that any segment which may be present on the chip is pushed off (towards the AgCl chip). The distance pushed must be less than the distance of the dry segment, i.e. 17*10 or 170 distance units. The current pushback distance is 150. While this is close to the dry distance, the dry segment is not push back so far due to the hysteresis (backlash) in the gear mechanism.

4.1.5 Analysis

At this point, a thin layer of analysis fluid is present in the walls of the conduit and on the sensors, and any ALP on the sensors will generate p-aminophenol which will be oxidized at the electrodes. The current is measured and if greater than 30 nA, the amp is switched to a low gain mode (ca. 160 pA/bit) so that if "pinned" (> 32 nA) in high gain mode (2 pA/bit), a meaningful current will be recorded.. The amp gain is recorded in SeqCounter[7]. After a delay of 5 seconds, the time is marked (index of amp data array at which TimeStamp function was executed, stored in SeqCounter[9]), and the conductivity is recorded and stored in SeqCounter[10]. After a further 3 s delay, the amp is switched to low gain for 3s then back to high gain. The plunger is retracted a fixed distance in order to check for following segments, and after collecting data for 3 s further, the cycle is completed.

4.1.5.1 Why Do We Use a Thin Film for Analysis?

Following capture/labeling and washing, we hope to end up with a sensor covered with microparticles to which are immobilized Ab-Antigen-Ab-ALP complexes. When we feed substrate (pAPP) to the ALP in this layer, p-aminophenol (pAP) is generated and can be oxidized at the electrode. The maximum current resulting from this oxidation will be realized only if all of the pAP generated in the layer is oxidized at the electrode. However, when pAP is initially formed, part of it diffuses towards the electrode where it is oxidized and part diffuses away from the sensor into the bulk of the solution. As the bulk of the solution becomes "back-filled" with pAP, the net loss of pAP to the bulk diminishes until a steady state is reached in which the amount of pAP generated by the enzyme per unit time is equal to

the amount oxidized at the electrode per unit time and the current achieves a steady state plateau. The time required to achieve steady state increases with the thickness of the fluid layer. If we were to perform the analysis with the sensor conduit filled with fluid, the time to steady state would be entirely impractical. However, by pulling the fluid off the chip so as to leave a thin film, steady state is achieved within 5 to 10 s. Provided that sufficient substrate is available, the steady state current will depend only on the coverage of ALP (and hence antigen) and not on film thickness or electrode area. At extreme ALP coverage (high antigen concentration in sample), currents are sometimes observed to decline before steady state is reached – this is due to depletion of the substrate.

4.1.6 Post-Sequence Calculations

Following the analysis phase of the cycle, the data can be manipulated as desired by means of standard C functions. At present, these calculations are fairly minimal. In the routine CondWashAnalysis, the minimum conductivity during the wash is determined from stored conductivity data – if the minimum conductivity is > 1000 bits, a SeqErrorCode = 1004 is set. The time at which the wash fluid arrived at the Hct bars is calculated. The routine SampleCondSizeAnalysis calculates the sample arrival time (at beginning of cycle), the sample conductivity in the first and second halves of the capture phase, the average time for the sample to traverse the Hct sensors during mixing in either direction, and the average time that the Hct bar is dry as the motor changes direction during the capture oscillations. Analysis of the Amp0 data involves calculating the average current in approx. 1s windows before and after the TimeStamp, and after the change to low gain (function MultiGain Analysis). The Amp1 data is averaged in the same 1s window but only before the TimeStamp (function Amp1Analysis). Coefficients are applied in the function ComputeTnIResult and the results are displayed.

Summary of Data Reported in the SeqCounter Data Array

SeqCounter[n]	Datum Name	Description
0	initSampleCond	initial sample conductivity
1	MixCount	# capture mix cycles
2	finalSampleCond	final sample conductivity
3	iSample (bits)	current in sample after capture
4	AvgTransitSC(msec)	transit time of sample towards

		sample chamber (SC)
5	AvgTransitWC(msec)	transit time of sample to waste waste chamber (WC)
6	tSampleArrival(msec)	sample arrival time
7	Gain(pA/bit)	gain during amp analysis
8	TotalDryMoves	# of moves to achieve dry sensor
9	tStampAmp	index of amp calculation
10	CondDuringAnalysis	conductivity during analysis
11	iA01(bits)	Amp0 current in first window
12	iA02bits)	Amp0 current in second window
13	iA0LoGain(bits)	Amp0 current low gain window
14	iA1(bits)	Amp1 current in first window
15	MinWashCond	Minimum conductivity during wash
16	tWet	Arrival time of analysis fluid
17	AvgDrySC(msec)	dry time on SC side (capture)
18	AvgDryWC(msec)	dry time on WC side (capture)
19	TnIx100 (ng/mL)	calculated [cTnI] * 100

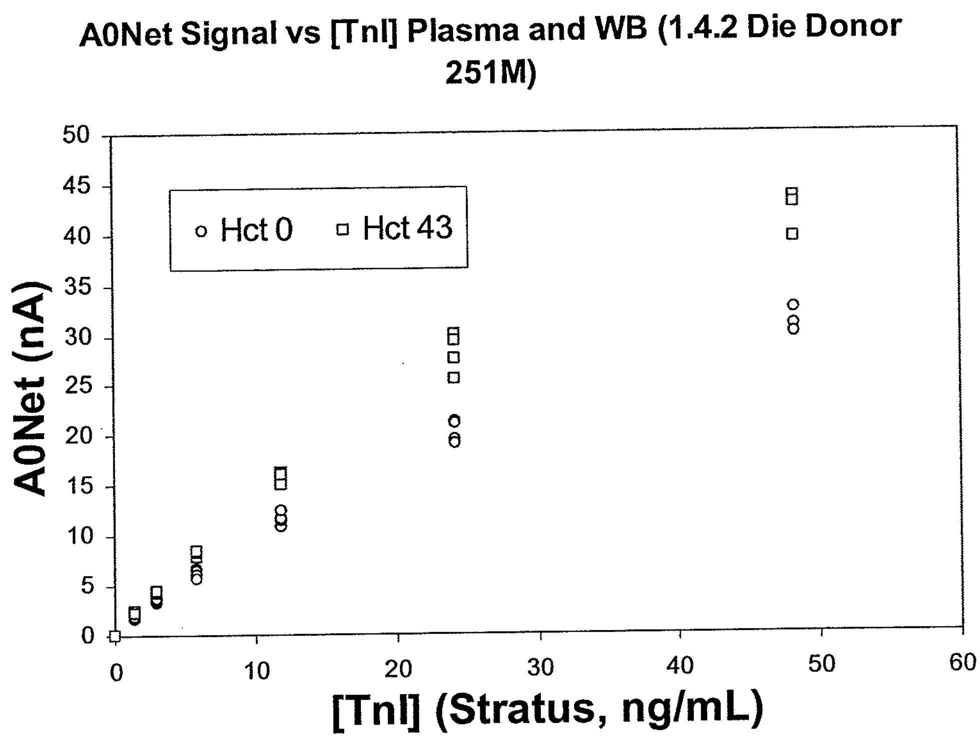
4.2 Algorithm For Calculation of [cTnI]

4.2.1 Calculation of Net Current

The net current is calculated as the difference between the analyte sensor, i.e. TnI sensor in this case, and the reference sensor, and then subtracting an offset current that is a constant characteristic of the two sensors:

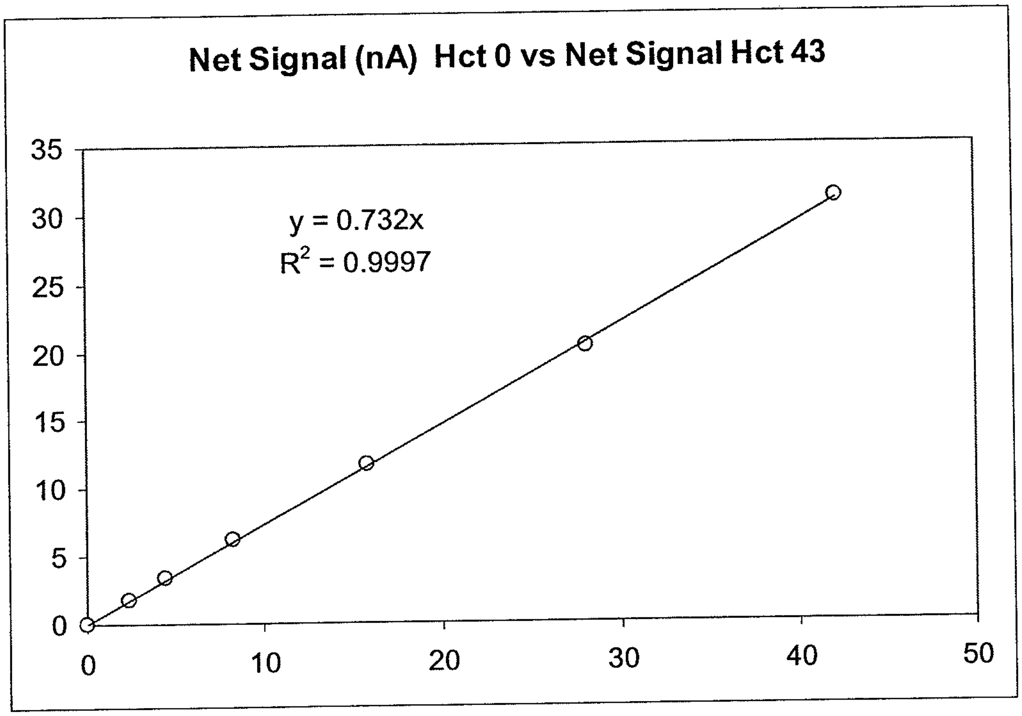
$$\text{iNet} = \text{iAmp0 (TnI)} - \text{iAmp1 (ref)} - \text{iOffset}$$

While the TnI sensor has a coating of antibodies which recognize cTnI specifically, the reference sensor has a coating of human serum albumin and will not bind cTnI specifically. Instead, the reference sensor may bind proteins non-specifically, e.g. Conjugate-ALP or cTnI-Conjugate-ALP which would give rise to non-specific ALP signal. Thus the reference sensor is simply a sensor of the level of non-specific binding of the conjugate. Non-specific binding can vary strongly from one sample to another and thus correction for this is essential for the realization of low detection limits. If one plots the net signal as a function of [cTnI], a non-linear function is evident. Such plots are illustrated below for plasma and whole blood.



4.2.2 Hematocrit Correction

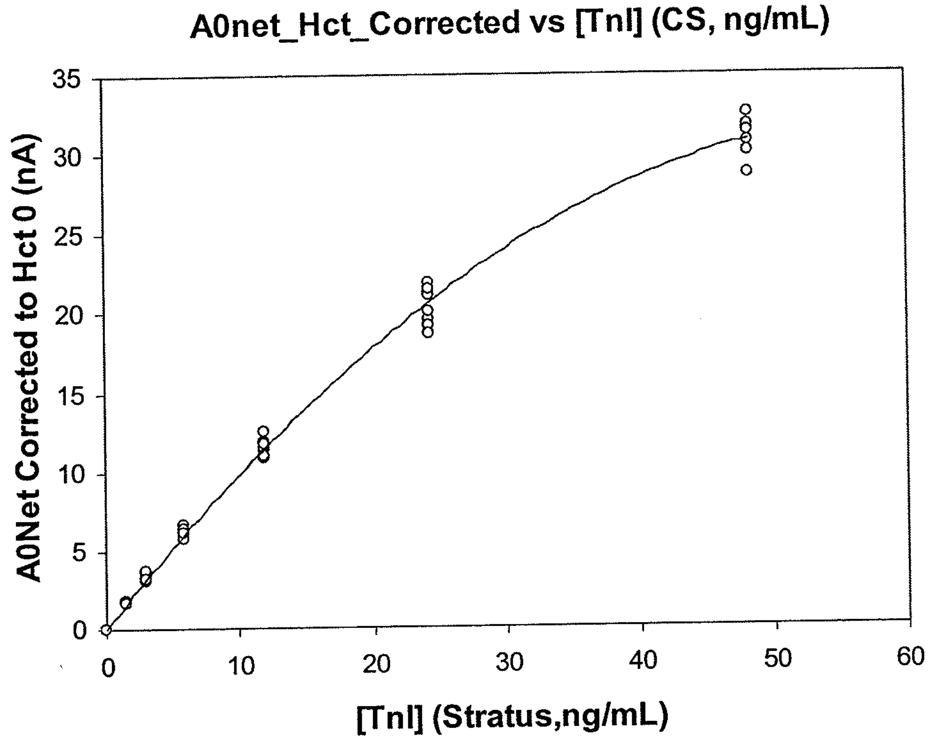
It's clear from the previous figure that sensor response is dependent on hematocrit. At least part of this dependence lies in the fact that the conjugate concentration increases linearly with hematocrit since a fixed amount of conjugate dissolves in a plasma volume which decreases linearly as hematocrit increases. Experimentally, it is observed that the TnI sensor signal increases linearly with hematocrit. If a sample of whole blood is spiked with cTnI, spun, and the red cell and plasma fractions are re-combined so as to generate a family of samples having *different hematocrits but the same plasma concentration of cTnI,* a plot of signal vs hematocrit will be linear. Further, if the experiment is repeated so as to generate signal vs Hct data for a range of [cTnI], the slopes and intercepts of the signal vs Hct data are themselves linear in [cTnI]. The result of this is that a simple linear Hct correction can be applied. For the data of the previous figure, if one plots the signal for each Hct 0 (plasma) sample against the signal for the nascent Hct (ca. 40) sample for each level of [cTnI], a linear plot results as illustrated below.



Determination of the Hct correction to be applied is straightforward (see Immuno448) and results in:

$$iNetHctCorr = iHct0 = iNetHct*(1\text{-}c0*Hct)$$

where c0 = s/Hct with s being the slope of the plot above and Hct being that of the sample employed in the plot. iNetHct is the net current observed for a sample with Hct = Hct. The result is that the current is corrected to the value that would be expected for a plasma sample. Application of the correction to the Current vs [cTnI] data above results in coalescence of the two curves as illustrated in the following figure.



4.2.3 Temperature Correction

The capture phase of the analysis is conducted with the chips heated to a constant temperature, currently 47 degrees – this temperature enhances mass transfer to the sensor yet is not so high as to compromise the reagent or analyte stability. However, because the sample is oscillated in the sensor conduit for the duration of the capture, the effective sample temperature is lower than the chip temperature setpoint. The ambient temperature in the vicinity of the cartridge has a significant effect on the effective temperature during capture. Experimentally, we observe that the signal generated is linearly proportional to base thermistor temperature . The magnitude of the effect is significant, amounting to something on the order of a 2% increase in signal per degree of temperature increase when the capture is carried out with chip heating to 47 degrees. Because of the linearity of the dependence, the temperature correction takes the same form as the hematocrit correction:

$$\text{iNet\_tCorr\_HctCorr} = \text{iNetCorr} = \{1 - \text{c1}*(23 - \text{Tbase})\}*\text{iNetHctCorr}$$

Tbase is the analyzer temperature measured at the base thermistor and c1 is the temperature correction coefficient as determined by the method in Section 5.3.7. All data are corrected to 23 degrees centigrade (the average temperature encountered in the field).

4.2.4 Calculation of [cTnI]

The shape of the iNetCorr vs [cTnI] curve illustrated in the previous figure is typical of the assay. The tendency of the slope to decrease at higher concentrations of cTnI, i.e. decreased sensitivity, represents a limiting behaviour in which the reagent set becomes saturated with analyte. Because there are fixed amounts of conjugate and capture antibodies during capture, and substrate during analysis, there must be a limit to the amount of analyte that can be captured and thus to the amount of current that can be generated. The iNetCorr vs [cTnI] curve tends asymptotically to such a limit. A conceptually similar situation is encountered in the study of enzyme kinetics. If a fixed amount of enzyme is exposed to increasing concentrations of substrate and the rate at which the substrate is converted to product is measured, a plot of rate vs [substrate] takes the same form as a plot of iNetCorr vs [cTnI]. There is a point at which the enzyme cannot turn over substrate any faster so that increasing the substrate concentration has no effect on the rate; the enzyme is said to be saturated with substrate; this leads to the Michaelis-Menten model of enzyme kinetics which predicts the asymptotic curve shape. Similarly, there is a point where increasing the concentration of analyte, cTnI, has no effect on the signal – the reagent set is saturated with analyte so that the system is now insensitive to further additions of analyte.

The Michaelis-Menten model results in the following equation relating reaction rate to substrate concentration:

$$V = \frac{\text{Vmax}[S]}{\text{Km} + [S]}$$

Here Km is the "Michaelis" constant, a characteristic of the enzyme/substrate combination and Vmax is the maximum velocity/rate that can be observed, i.e. the asymptotic velocity. If we re-cast the Michaelis-Menten equation in terms of iNetCorr vs. [cTnI], we have:

$$\text{iNetCorr} = \frac{\text{imax}[\text{cTnI}]}{\text{Km} + [\text{cTnI}]}$$

where imax is now the maximal current that can be generated, i.e. current corresponding to the maximum amount of labeled-bound cTnI on the sensor, and Km is a constant determined by the reagent set. It can be seen that if [cTnI] is much smaller than Km, then the net current is linearly proportional to [cTnI], i.e. iNetCorr = imax[cTnI]/Km. Consideration of this

limiting case leads to the realization that imax = Km*S where S is the slope of the curve (nA/ng/mL) at low [cTnI]. At the other extreme, if [cTnI] is much greater than Km, iNetCorr tends to imax. Taking the reciprocal of the above equation leads to the Lineweaver-Burk form:

$$\frac{1}{\text{iNetCorr}} = \frac{1}{S} * \frac{1}{[\text{cTnI}]} + \frac{1}{\text{Km*S}}$$

A plot of 1/iNetCorr vs 1/[cTnI] will be linear with slope 1/S and intercept 1/(Km*S).

Though a Michaelis-Menten function of the form above can be fit to the iNetCorr vs [cTnI] data, a polynomical can also be employed. In practice, it is often found that the Michaelis-Menten form fits well at the low end ([cTnI]) but the high end is better modeled using a polynomial. In this case, both functions may be employed simultaneously with the transition occuring at a fixed current level. That the MM treatment does not always fit well at high [cTnI] may stem from the fact that while saturation of the enzyme model stems from a single source, i.e. the finite amount of enzyme, multiple sources including conjugate concentration, capture antibody coverage, and substrate concentration during analysis may all contribute to saturation of the antibody reagent set. Suffice it to say that a combination of Michaelis-Menten and polynomial fitting can be used to model the data and afford a route for calculation of analyte concentration.

5. Finished Goods Protocol

One area of considerable concern and attention is the design of a finished goods protocol to be employed for the cTnI cartridge. This protocol must be reasonably straightforward to carry out, yet afford coefficients that will encompass a sufficient representation of the clinical patient population to be encountered in the field. This is not as simple as in the case of a glucose or oxygen assay since the immunoassay is inherently more sensitive to immunological and other attributes which may vary from one sample/patient to another.

Week to week activities of the immunoassay development team include the fabrication and testing each week of a "mainline" build by the SAL (semi-automated line) team. This build incorporates the most current cartridge, sensor, reagent and software designs. These cartridges are subjected to finished goods testing, are employed in many day

EXHIBIT C

[redacted] Set up for Conjugate on Chip drying Experiment

use 02013i 15µL + 6µL 0.05% Tween 20 in D.H2O

+ 2.8 mg Lactitol. (put 6µL in Lactitol & Dissolve)
Then add 2 x 7.5µL conjugate
So its 15% solids dil 1:4 44 pulse 0.50 pressure

IH-18 Wafer aHSA beads (#1) on Pot 6) overcoat [redacted]
aTnI C4 beads on Amp 1
Pot 5 & Amp 0 blank

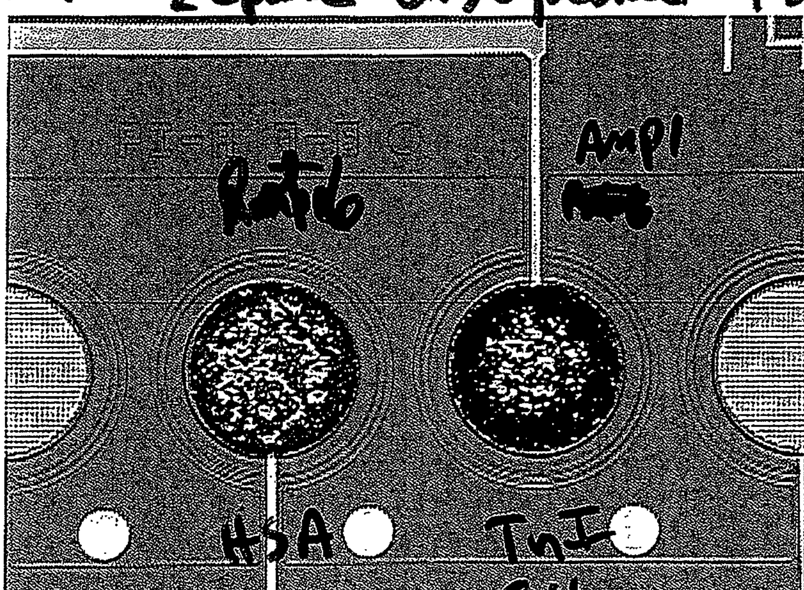
Conjugate Clear 660µ x 19µ

~500 prints from 20µL or 40nL per print

3.25nL dry ⇒ 22nL by Solids

So assume 30nL 800U/mL original composite 0.024U OK
*1.4
\\
0.017U OK

IH-19 22 pulse 0.50 pressure 1 suck back



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